REPORT

96-HOUR ACUTE TOXICITY STUDY IN CARP

WITH

[redacted]

(SEMI-STATIC)

NOTOX Project 338761
NOTOX Substance 111834/B

CONFIDENTIALITY STATEMENT

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STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Study Director
Ing. M.H.J. Migchielsen

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Date: November 13, 2002

Management:
Ing. E.J. van de Waart M.Sc.
Head of Genetic & Ecotoxicology

.....

Date: 15/11/2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/AUDITS	REPORTING DATES
on-site inspection(s) (Process)	
July 08 to 15, 2002 (Ecotoxicology) August 19 to 30, 2002 (Analytical support)	July 17, 2002 September 02, 2002
protocol inspection(s) (Study)	
July 04, 2002	July 04, 2002
report audit(s) (Study)	
November 04, 2002	November 04, 2002

Head of Quality Assurance C.J. Mitchell B.Sc.

Date: 18-NOV-02.

SUMMARY

96-Hour Acute Toxicity Study in carp with [redacted]

The study procedures described in this report were based on the EEC directive 92/69; Part C: methods for the determination of ecotoxicity, Publication No. L383, December 1992, C.1. "Acute toxicity for fish", and the OECD guideline No. 203: "Fish Acute Toxicity Test", Adopted 17 July, 1992.

The batch of [redacted]ested was a clear and colourless liquid consisting of two main components, i.e. 28.6% peroxidic compounds and 67% Dimethyl phtalate. [redacted] was completely miscible with test medium at the concentrations tested.

The project started with a static range-finding test exposing three carp per test group to nominal [redacted] concentrations of 0.1, 1.0, 10 and 100 mg/l. All fish exposed to nominally 100 mg/l died within 3 hours of exposure. No mortality was observed at the lower test concentrations. Hence, the 96h-LC_{50} for carp exposed to [redacted] was expected to be between 10 and 100 mg/l.
Analytical results showed that the measured concentrations of both main components decreased by more than 20% during the test period. It was decided to continue testing applying a semi-static test design with daily renewal of test solutions as concentrations did not decrease by more than 20% during the first 24-hour test period.

The project was continued with a final LC_{50} study exposing seven carp per concentration to [redacted] concentrations ranging from 10 to 100 mg/l, increasing with a factor of 1.8. All test solutions were daily renewed. Samples for analysis were taken at the start and after 72 hours of exposure from freshly prepared solutions and from 24-hour old test solutions at 24 and 96 hours of exposure.

Analysis of the samples taken during the final test showed that the measured concentrations (based on both components) were in agreement with nominal in the freshly prepared solutions at the start of exposure (88-101%) and the freshly prepared solutions at 72 hours of exposure (93-99%). This indicated that preparation procedures were adequate and repeatable.
During the 24-hour periods between renewals the concentrations measured did not decrease by more than 20% below initial. In addition, the average exposure concentrations all remained above 80% relative to nominal. Consequently, the calculated toxicity parameters were based on the nominal test concentrations.
In the control group no fish died, and all test conditions (pH, oxygen and temperature) remained within the ranges prescribed by the protocol.

[redacted] induced no mortality in carp at or below nominally 10 mg/l.

The 3¾h-LC_{50} was 60 mg/l with a 95% confidence interval between 52 and 81 mg/l.

The 24h-LC_{50} was estimated to be 21 mg/l with 14% mortality at 18 mg/l and 100% mortality at 32 mg/l (regression line: log10(conc.)=1.01+(probit-2.61)/7.73).

The 96h-LC_{50} was already reached within 48 hours of exposure being 16 mg/l with a 95% confidence interval between 14 and 21 mg/l.

Based on this study [redacted] is classified as harmful to aquatic organisms according to the EEC-directive 98/98.

PREFACE

Sponsor	[redacted]
Study Monitor	Dr. C.L.J. Braun SHERA, Regulatory Affairs
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Aquatic Toxicology: Study Director Technical Coordinator	 Ing. M.H.J. Migchielsen Mrs. E. Mutsaards
Analytical Chemistry: Principal Scientist	 Dr. Ir. E. Baltussen
Study Plan	Start of project: July 04, 2002 Start of range-finding test: July 22, 2002 Completion of final test: September 13, 2002 Completion of analysis: September 27, 2002 Draft report: November 06, 2002 Completion of project: November 13, 2002

TEST SUBSTANCE

Identification Chemical name CAS RN	[redacted]
Description	Clear colourless liquid
Batch	1510-14
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2003
Density	Approx. 1160 $kg.m^{-3}$
Stability in water	Unknown

The sponsor is responsible for all test substance data unless determined by NOTOX.

PURPOSE

The purpose of the study was to evaluate the test substance for its ability to generate acute toxic effects in *Cyprinus carpio* during an exposure period of 96 hours and, if possible, to determine the LC_{50} at all observation times.

GUIDELINES

The study procedures described in this report were based on the following guidelines:

European Economic Community (EEC), EEC directive 92/69, Part C: Methods for the determination of ecotoxicity, Publication No. L383, December 1992, C.1. "Acute toxicity for fish".

The OECD guidelines for Testing of Chemicals, guideline No. 203: "Fish Acute Toxicity Test", Adopted 17July, 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data. No data will be withdrawn without the sponsor's written consent.

DEFINITIONS

Fish were considered to be dead when no reaction was observed after touching the caudal peduncle and visible breathing movements were absent.

The LC_{50} is the concentration killing 50% of the fish after a defined period of exposure.

TEST SYSTEM

Species	Carp (*Cyprinus carpio*, Teleostei, Cyprinidae) (Linnaeus, 1758)
Source	Zodiac, proefacc, "De Haar Vissen", L.U. Wageningen, the Netherlands.
Mean length	Static range-finding test: 2.5 ± 0.16 cm Semi-static final test: 2.1 ± 0.18 cm
Mean weight	Static range-finding test: 0.50 ± 0.12 g Semi-static final test: 0.13 ± 0.04 g
Characteristics	F1 from a single parent-pair bred in UV-treated water.
Reason for selection	This system has been selected as an internationally accepted species.
Total fish used	54

HOLDING

Quarantine/Acclimatisation	At least 12 days after delivery.
Medium	ISO-medium, formulated using Milli-Ro water (tap-water purified by reverse osmosis; Millipore Corp., Bedford, Mass., USA) with the following composition: Ca^{2+} 80 mg/l Mg^{2+} 12 mg/l Na^{+} 15 mg/l K^{+} 3 mg/l Cl^{-} 145 mg/l SO_4^{2-} 49 mg/l HCO_3^{-} 47 mg/l Hardness is 250 mg $CaCO_3$/l
Measurements	pH, nitrate and nitrite concentration and ammonia concentration: once a week. Temperature: every day.
Feeding	Daily with Trouvit.
Control of sensitivity	A reference test with pentachlorophenol (PCP, SIGMA) is carried out once a year. The results of the most recent performed test are appended to the report.
Validity of batch	In the batch of fish used for the test, mortality during the seven days prior to the start of the test was less than 5%.

PREPARATION OF TEST SOLUTIONS

The standard test procedures required generation of test solutions, which contain completely dissolved test substance concentrations or stable and homogeneous mixtures or dispersions. The testing of concentrations that disturbed the test system were prevented (e.g. film of the test substance on the water surface).

The batch of [redacted] tested was a clear and colourless liquid consisting of two main components, i.e. 28.6% peroxidic compounds and 67% Dimethyl phtalate (see also attached analysis certificate). [redacted] was completely miscible with test medium at the concentrations tested.

Preparation of test solutions started with stock solutions at nominally 100 mg/l. These solutions were magnetically stirred for 15 to 20 minutes following treatment with ultrasonic waves for 5 minutes during the range-finding test. The resulting, clear and colourless, stock solutions were then used to prepare the lower test concentrations by subsequent dilutions in test medium. The test solutions were daily renewed during the final test. Part of the solutions was used for testing with Daphnia magna (NOTOX Project 338772).

STATIC RANGE-FINDING TEST

A range-finding test was performed to provide information about the range of concentrations to be used in the final test: three fish per concentration were exposed to a concentration range of 0.1 to 100 mg/l with an increasing factor of 10. Samples for analysis were taken from 1.0 and 10 mg/l.

Sampling: Frequency	at t=0 h, t=24 h and t=96 h.
Volume	12 ml from the approximate centre of the test vessel.
Storage	All samples were stored in a freezer until analysis.

SEMI-STATIC FINAL TEST:

TEST CONCENTRATIONS

Nominal test concentrations	10, 18, 32, 56 and 100 mg/l.
Blank-control	Test medium without test substance or other additives (0 mg/l).

TEST PROCEDURE AND CONDITIONS

Test duration	96 hours
Test type	Semi-static, with renewal of test solutions after each 24-hour test period.
Test vessels	5.5 litres, all-glass.
Test medium	ISO-medium, aerated until the dissolved oxygen concentration had reached saturation and the pH had stabilised. After aeration the hardness was 250 mg $CaCO_3$ per litre and the pH was 7.6-7.7.
Number of fish	7 fish per concentration and control.
Loading	0.23 g fish/litre, i.e. 7 fish per 4 litres of test medium.
Illumination	16 hours photoperiod daily.
Aeration	The test media were not aerated during the test.
Feeding	No feeding from 48 hours prior to the test and during the total test period.
Introduction of fish	Directly after preparation of the test media.
Euthanasia	At the end of the test the surviving fish were rapidly killed by exposing them to ca. 1.2% ethylene glycol monophenylether in water.

SAMPLING FOR ANALYSIS OF TEST CONCENTRATIONS

During the final LC_{50} test samples for analysis were taken from the approximate centre of the test vessels according to the following sampling schedule:

Sampling: Frequency	At t= 0 h from freshly prepared and at t= 24 h from 24h-old solutions of all test concentrations. At t =72 h from freshly prepared and at t=96 h from 24h-old solutions from 0, 10 and 18 mg/l.
Volumes	0, 10 and 18 mg/l: 6 ml 32 mg/l: 3 ml 56 mg/l: 2 ml 100 mg/l: 1 ml
Storage	Samples were stored in a deep-freeze until analysis together with freshly taken samples at t=96 hours.

Additionally, reserve samples of 12 ml were taken from all test solutions at t=0 and t= 24 h and from the test concentrations with surviving fish at t=72 and t=96 h for possible analysis. If not already used, these samples were stored in a freezer for possible analysis until delivery of the final report with a maximum of three months. The method of analysis and specification of the samples analysed is described in the appended Analytical Report.

MEASUREMENTS AND RECORDINGS

Mortality and other effects	At 3¾, 24, 48, 72 and 96 hours following the start of exposure. Dead fish were removed when observed.
Fish length and weight	Ten fish of the batch used for the test, were weighed and measured prior to the start of the test.
Dissolved oxygen content Temperature and pH	Daily in all vessels, beginning at the start of the test (day 0).

DATA HANDLING

The LC_{50} was determined using:

the maximum likelihood estimation method with the probits of the percentages of dead fish as function of the logarithms of the corresponding concentrations (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition)

RESULTS

Static range-finding test:

Table 1 shows the mortality observed during the static range-finding test.

All fish exposed to nominally 100 mg/l died within 3 hours of exposure. No mortality or other effects were observed at the lower test concentrations. Hence, the 96h-LC_{50} for Carp exposed to [redacted] was expected to be between 10 and 100 mg/l.

Table 1: Incidence of mortality and total mortality during the range-finding test.

Concentration TRIGONOX R-938 Nominal (mg/l)	Initial number of fish	Cumulative mortality					Total Mortality (%)
		3h	24h	48h	72h	96h	
0.1	3	0	0	0	0	0	0
1.0	3	0	0	0	0	0	0
10	3	0	0	0	0	0	0
100	3	3	3	3	3	3	100

Stability of [redacted] under test conditions

Analysis of actual [redacted] concentrations was based on the two major components present in [redacted] (indicated as MIPKP-T3 peak 1 and MIPKP-T3 peak 2). The results showed that the measured concentrations of both components decreased by more than 20% during the test period (see Tables 1 and 2 of the appended Analytical report). It was decided to continue testing applying a semi-static test design with daily renewal of test solutions as concentrations did not decrease by more than 20% during the first 24-hour test period.

Final study:

Measured concentrations

The results of analysis of the samples taken during the final study are described in Tables 3 and 4 of the appended Analytical Report.

Analysis of the samples taken during the final test showed that the measured concentrations (based on both components) were in agreement with nominal in the freshly prepared solutions at the start of exposure (88-101%) and the freshly prepared solutions at 72 hours of exposure (93-99%). This indicated that preparation procedures were adequate and repeatable.
During the 24-hour periods between renewals the concentrations measured did not decrease by more than 20% below initial. In addition, the average exposure concentrations all remained above 80% relative to nominal. Consequently, the calculated toxicity parameters were based on the nominal test concentrations.

Mortality and other effects

The mortality data are presented in Table 2. Table 3 specifies the clinical effects observed at the different test concentrations.

The results of the final test were in agreement with the result of the range-finding test and allowed for a reliable determination of the toxicity parameters.

Table 2: Incidence of mortality and total mortality during the final test.

Concentration [redacted] (mg/l)	Initial number of fish	Cumulative mortality					Total Mortality (%)
		3¾h	24h	48h	72h	96h	
Blank-control	7	0	0	0	0	0	0
10	7	0	0	0	0	0	0
18	7	0	1	5	5	5	71
32	7	0	7	7	7	7	100
56	7	2	7	7	7	7	100
100	7	7	7	7	7	7	100

Table 3: Clinical effects observed during the final test.

Concentration [redacted] (mg/l)	Time of recording (hours)	Specification of effects	Relative number
Blank-control	0-96	No abnormalities	7/7
10	0-48	No abnormalities	7/7
	72-96	Discoloured	7/7
18	0-3¾	No abnormalities	7/7
	24	Swimming at the surface and discoloured	6/6
	48	Discoloured	2/2
	72-96	Swimming at the surface and discoloured	2/2
32	3¾	Swimming at the surface and discoloured	7/7
56	3¾	Immobile and discoloured	5/5

Experimental conditions

The results of measurement of pH and oxygen concentrations are presented in Tables 4 and 5, respectively. The results of measurement of the temperature in the various test solutions is presented in Table 6.
The temperature in the blank-control was continuously measured and maintained between 21.3 and 21.8°C during the 96-hour test period.

Table 4: pH-values measured during the final test.

Concentration [redacted] (mg/l)	Day 0	Day 1		Day 2		Day 3		Day 4
	Fresh	Fresh	Old	Fresh	Old	Fresh	Old	Old
Blank-control	7.7	7.7	7.7	7.7	7.4	7.7	7.5	7.7
10	7.7	7.7	7.5	7.7	7.5	7.7	7.5	7.7
18	7.7	7.7	7.5	7.8	7.6	7.7	7.6	7.7
32	7.7		7.7					
56	7.7		7.7					
100	7.7							

Table 5: Dissolved oxygen concentrations (mg/l) measured during the final test.

Concentration [redacted] (mg/l)	Day 0	Day 1		Day 2		Day 3		Day 4
	Fresh	Fresh	Old	Fresh	Old	Fresh	Old	Old
Blank-control	8.6	8.5	7.5	8.8	7.8	8.9	8.0	8.1
10	8.6	8.6	7.7	8.8	7.9	9.0	8.0	8.2
18	8.6	8.7	7.8	8.8	8.2	8.9	8.5	8.6
32	8.6		8.5					
56	8.6		8.6					
100	8.7							

Table 6: Temperatures (°C) measured during the final test.

Concentration [redacted] (mg/l)	Day 0	Day 1		Day 2		Day 3		Day 4
	Fresh	Fresh	Old	Fresh	Old	Fresh	Old	Old
Blank-control	21.4	20.9	21.4	21.4	21.7	21.3	21.5	21.6
10	21.4	21.0	21.3	21.4	21.7	21.3	21.5	21.8
18	21.4	21.0	21.4	21.4	21.6	21.3	21.6	21.8
32	21.4		21.3					
56	21.4		21.4					
100	21.4							

ACCEPTABILITY OF THE TEST

1. No mortality was observed in the control group.
2. The analytical program provided clear evidence that the actual concentrations during the 24-hour periods between refreshment had been maintained at more than 80 % of the initial concentrations and in agreement with nominal.
3. Further, all test conditions (pH, oxygen concentration and temperature) remained within the ranges prescribed by the protocol.

CONCLUSIONS

Under the conditions of the present test [redacted] induced no mortality in carp at or below nominally 10 mg/l.

The 3¾h-LC_{50} was 60 mg/l with a 95% confidence interval between 52 and 81 mg/l.

The 24h-LC_{50} was estimated to be 21 mg/l with 14% mortality at 18 mg/l and 100% mortality at 32 mg/l (regression line: log10(conc.)=1.01+(probit-2.61)/7.73).

The 96h-LC_{50} was already reached within 48 hours of exposure being 16 mg/l with a 95% confidence interval between 14 and 21 mg/l.

Table 7: 3¾h-LC_{50} values and related parameters.

3.75h-LC50 Carp = 60.2 mg/l
95 % fiducial limits: 51.6 - 81.3 mg/l

index of regression significance: g=0.10
chi-squared=0.69, with 1 degrees of freedom

regression line: log10(conc.)=1.51+(probit-2.85)/7.89

conc. mg/l	group size	mortality	corrected fraction	expected fraction	chi2
32	7	0	0.00	0.00	0.00
56	7	2	0.29	0.40	0.39
100	7	7	1.00	0.96	0.30
					0.69

Figure 1: Percentage of mortality of fish as a function of the log concentration after 3¾ hours of exposure.

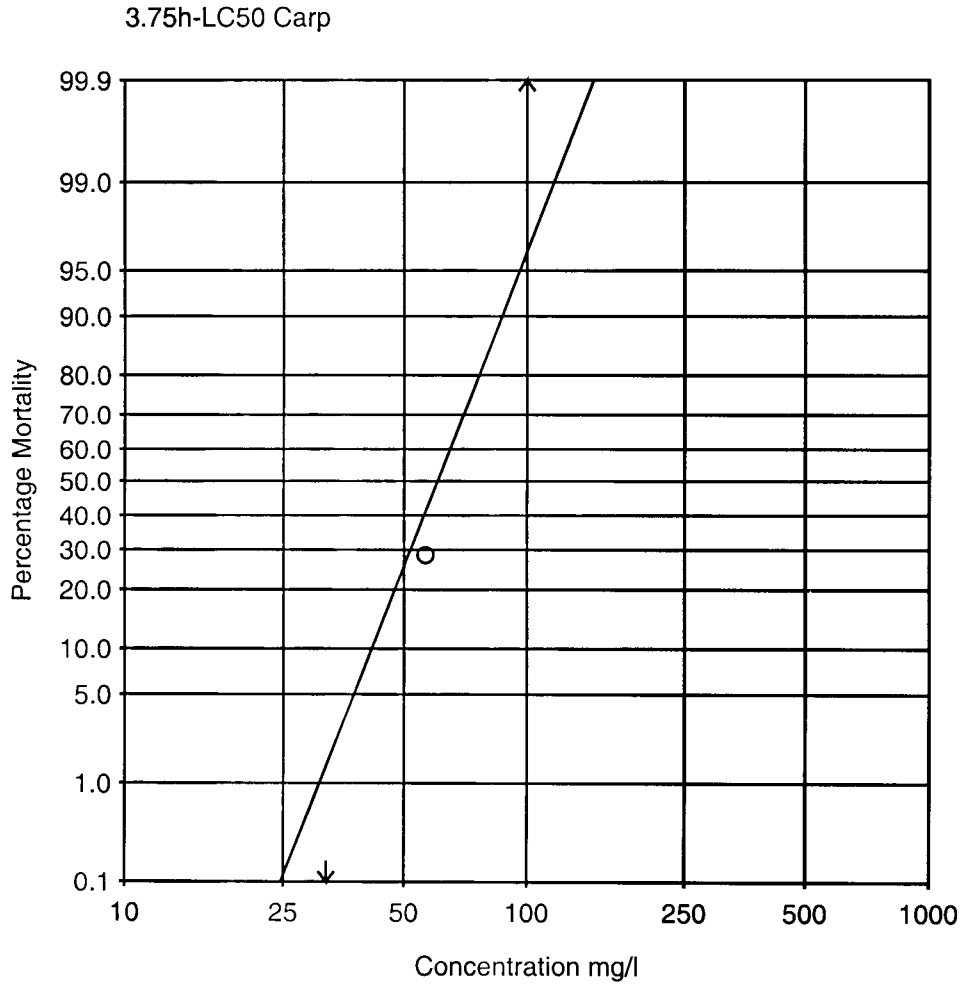


Table 8: 24h-LC_{50} values and related parameters.

24h-LC50 Carp = 20.7 mg/l

heterogeneous data, h=1.55
index of regression significance: g=5.53
chi-squared=1.55, with 1 degrees of freedom

regression line: log10(conc.)=1.01+(probit-2.61)/7.73

conc. mg/l	group size	mortality	corrected fraction	expected fraction	chi2
10	7	0	0.00	0.00	0.00
18	7	1	0.14	0.32	1.01
32	7	7	1.00	0.93	0.54
					1.55

Figure 2: Percentage of mortality of fish as a function of the log concentration after 24hours of exposure.

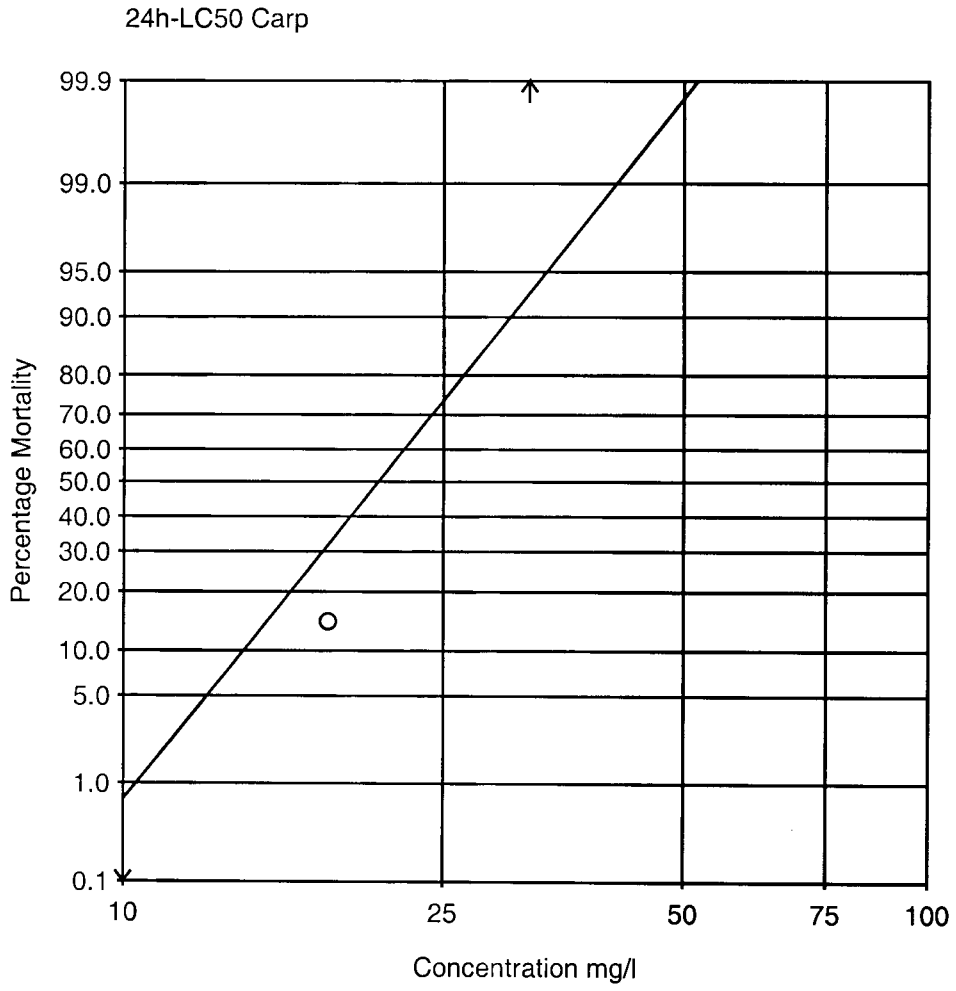


Table 9: 48h-LC_{50} values and related parameters.

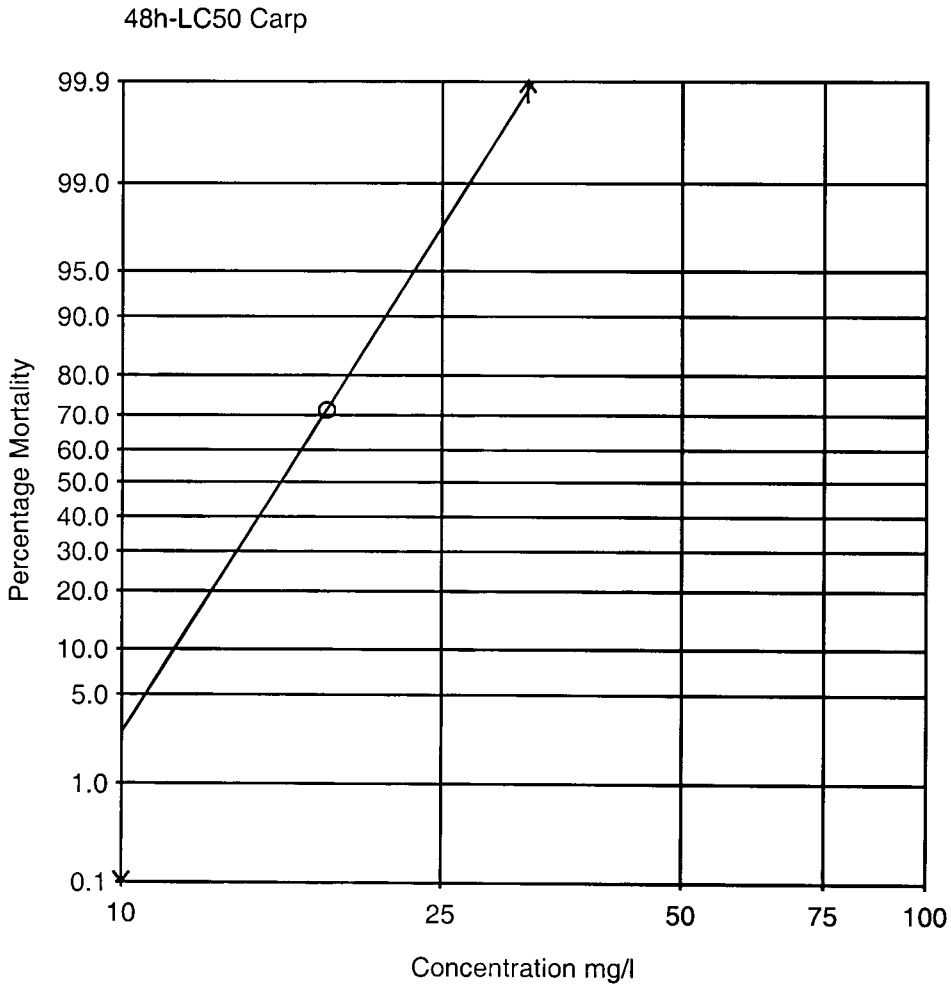
48h-LC50 Carp = 15.7 mg/l
95 % fiducial limits: 13.9 - 20.7 mg/l

index of regression significance: g=0.14
chi-squared=0.01, with 1 degrees of freedom

regression line: log10(conc.)=1.00+(probit-3.09)/9.78

conc. mg/l	group size	mortality	corrected fraction	expected fraction	chi2
10	7	0	0.00	0.00	0.00
18	7	5	0.71	0.72	0.00
32	7	7	1.00	1.00	0.01
					0.01

Figure 3: Percentage of mortality of fish as a function of the log concentration after 48 hours of exposure.



REFERENCE TEST

96-hour acute toxicity study in the carp with PCP; NOTOX Project 344611 (Batch 02-01)

The study procedures described in this report were based on the EEC directive 92/69, Part C.1. "Acute toxicity for fish"; and the OECD guideline No. 203: "Fish Acute Toxicity Test", Adopted 17 July, 1992.

Start: 21-01-2002
End: 08-02-2002

This reference test was carried out to check the sensitivity of the test system as used by NOTOX. The reference substance was pentachlorophenol (PCP, SIGMA, Art. P9441, Batch 103H3488).

Concentrations: 0.06, 0.1, 0.15, 0.22 and 0.32 mg/l in ISO-medium.
Control: ISO-medium without test substance.

Incidence of mortality observed in the reference study:

Concentration PCP (mg/l) Nominal	Initial Number Of fish	Cumulative number of dead fish recorded at various time points after start of exposure					Total Mortality (%)
		2h	24h	48h	72h	96h	
Control	5	0	0	0	0	0	0
0.06	5	0	0	0	0	0	0
0.1	5	0	0	0	0	0	0
0.15	5	0	0	0	0	0	0
0.22	5	0	2	2	2	4	80
0.32	5	0	5	5	5	5	100

During the test the pH, oxygen concentration and the temperature of the medium were within the optimal ranges for fish.

Under the conditions of the present test PENTACHLOROPHENOL induced no lethal effects in carp at or below 0.15 mg/l. The 96h-LC_{50} for carp exposed to PCP was 0.20 mg/l (95 % confidence interval between 0.18 and 0.24 mg/l) with 100% mortality at 0.32 mg/l. The 24h-LC_{50} was 0.22 mg/l (95% confidence interval between 0.20 and 0.29 mg/l), and remained unchanged until 72h. The range of the 96h-LC_{50} for carp is generally between 0.10 and 0.46 mg/l based on historical data of reference tests performed approximately every 3 months from April 1988 until the end 2000, and annually since then. The response observed in carp originating from the present batch falls within this range.

The raw data and report from this study are kept in the NOTOX archives. The test described above was performed under GLP-conditions with a QA-check.

CERTIFICATE OF ANALYSIS

[redacted]

Certificate of Analysis

[redacted]

ICS-331

Product name : [redacted]

Chemical name : [redacted]

Batch number : **1510-14**

Test results:

Method	Analysis of	Unit	Result *1
Jo/72.11, Jo/95.2	Peroxidic compounds (sum) ***See page 2 for a specification***	% m/m	28.6 (± 1.5)
J20010792	[redacted]	% m/m	67.0 (± 1.0)
J20010792		% m/m	2.0 (± 0.3)
Amp/88.9	Water	% m/m	2.6 (± 0.3)
J20010792	Unidentified impurities	% m/m	0.5 (± 0.2)

*1 bracketed values are estimated 95% confidence intervals

File code : **TNA-2001007**

Analytical documentation : 20010792

[redacted]

CERTIFICATE OF ANALYSIS

[redacted]

Certificate of Analysis

[redacted]

[redacted]batch 1510-14: specification of the peroxidic compounds

structure	% m/m
[redacted]	

[redacted]

ANALYTICAL REPORT

96-HOUR ACUTE TOXICITY STUDY IN CARP

WITH

[redacted]

(SEMI-STATIC);

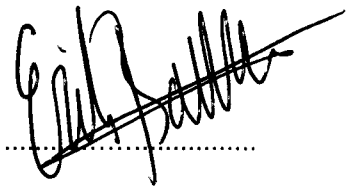
DETERMINATION OF THE CONCENTRATIONS

NOTOX Project 338761
NOTOX Substance 111834/B

REPORT APPROVAL

PRINCIPAL SCIENTIST:

Dr. Ir. E. Baltussen
(Analytical Chemistry)



Date: 07 NOV 2002

PREFACE

Study plan (analytical study)	Start: 26 July 2002 Completed: 27 September 2002

PURPOSE

The purpose of the analytical study was to determine the test concentrations.

REAGENTS

Acetonitrile	HPLC-grade, Labscan, Dublin, Ireland
Milli-Q water	Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges; Millipore, Bedford, MA, USA
ISO-medium	see main report

SAMPLE PRETREATMENT

All samples not analysed on the sampling day were stored in a deep freeze. On the day of analysis, the frozen samples were defrosted at room temperature.

The entire volume of each sample was transferred quantitatively into a 6 ml vial. If necessary, the vials were filled up to 6 ml with ISO-medium to obtain concentrations within the calibration range.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC CONDITIONS

Quantitative analyses were based on the area of two peaks (MIPKP-T3 peak 1 and MIPKP-T3 peak 2) with retention times of 13.6 and 14.5 minutes in the HPLC chromatogram of Trigonox R-938 (See NOTOX Project 352968: "Implementation and validation of an analytical method for Trigonox R-938").

Analytical conditions

A SPE-LC method was implemented and validated under Notox Project 352968. This method was based on a Zorbax RX-C18 column using a gradient of acetonitrile and Milli-Q water as the mobile phase, a column temperature of 25°C and a spectrophotometric detector set to read the absorbance at 220 nm.

Standard and calibration solutions

Standard solutions of Trigonox R-938 were prepared in acetonitrile.

Calibration solutions in ISO-medium were made up from two standard solutions.

DATA HANDLING

General

Mean:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

where
x_i = measured value
n = number of measurements

Maximum deviation: [(highest value - lowest value)/mean] * 100%
where 'mean' is the mean value of the highest and the lowest value.

Calibration

Response: R = Peak area test substance [units]

Calibration curve: The response was correlated with the concentration test substance, using linear regression analysis (least squares method). If necessary a weighting factor (1/concentration) was used.

R = a * C + b

R = response calibration solution [units]
C = concentration of test substance in calibration solution [mg/l]
a = slope [units*l/mg]
b = intercept [units]

During the range-finding test, a calibration curve was constructed using five concentrations. During the final test, calibration curves were constructed using six concentrations. For each concentration, two responses were used. The coefficient of correlation was > 0.99.

Samples

Concentration of [redacted] analysed in the samples:

$$C = \frac{(R-b) * d}{a} \text{ [mg/l]}$$

R = response sample [units]
d = dilution factor
a = slope [units*l/mg]
b = intercept [units]

Relative to nominal concentration:

$$\frac{\text{Concentration analysed}}{\text{Concentration nominal}} * 100\ [\%]$$

RESULTS

Tables 1-4 show the analytical results of this study*.

Table 1 Concentrations in test medium based on MIPKP-T3 peak 1 (range-finding test).

Time of sampling [hours]	Date of sampling [dd-mm-yy]	Date of analysis [2] [dd-mm-yy]	Concentration		
			Nominal [mg/l]	Analysed [1] [mg/l]	Relative to nominal [%]
0	22-06-02	06-08-02	1	2.11 [3]	211 [3]
			10	9.79	98
24	23-06-02	06-08-02	1	n.d.	n.a.
			10	9.33	93
96	26-06-02	06-08-02	1	n.d.	n.a.
			10	7.25	73

[1] Mean of duplicate samples. The maximum deviation between the responses was calculated for each sample and was < 10%.

[2] Samples were frozen until analysis.

[3] This peak was caused by a baseline disturbance, which probably does not correspond to test substance.

n.d. Not detected.

n.a. Not applicable.

Table 2 Concentrations in test medium based on MIPKP-T3 peak 2 (range-finding test).

Time of sampling [hours]	Date of sampling [dd-mm-yy]	Date of analysis [2] [dd-mm-yy]	Concentration		
			Nominal [mg/l]	Analysed [1] [mg/l]	Relative to nominal [%]
0	22-06-02	06-08-02	1	1.87 [3]	187 [3]
			10	9.35	94
24	23-06-02	06-08-02	1	n.d.	n.a.
			10	8.50	85
96	26-06-02	06-08-02	1	n.d.	n.a.
			10	4.80	48

[1] Mean of duplicate samples. The maximum deviation between the responses was calculated for each sample and was < 10%.

[2] Samples were frozen until analysis.

[3] This peak was caused by a baseline disturbance, which probably does not correspond to test substance.

n.d. Not detected.

n.a. Not applicable.

* All recoveries and relative values were calculated using not-rounded concentrations. Therefore, some differences might be observed when calculating the recoveries and relative values using the concentrations as mentioned in the tables.

Table 3 Concentrations in test medium based on MIPKP-T3 peak 1 (final test).

Time of sampling [hours]	Date of sampling [dd-mm-yy]	Date of analysis [dd-mm-yy]	Concentration		
			Nominal [mg/l]	Analysed [1] [mg/l]	Relative to nominal [%]
0 (fresh)	09-09-02	13-09-02 [2]	0	n.d.	n.a.
			10	8.81	88
		26-09-02 [2]	18	16.1	89
			32	28.5	89
			56	50.9	91
			100	92.0	92
24(old)	10-09-02	13-09-02 [2]	0	n.d.	n.a.
			10	8.51	85
			18	13.4	74
			32	24.8	77
			56	56.4	101
72 (fresh)	12-09-02	13-09-02 [2]	0	0.066	n.a.
			10	9.25	93
			18	16.7	93
96 (fresh)	13-09-02	13-09-02	0	1.85	n.a.
			10	8.12	81
			18	15.5	86

[1] Mean of duplicate samples. The maximum deviation between the responses was calculated for each sample and was < 10%.

[2] Samples were frozen until analysis.

n.d. Not detected.

n.a. Not applicable.

Table 4 Concentrations in test medium based on MIPKP-T3 peak 2 (final test).

Time of sampling [hours]	Date of sampling [dd-mm-yy]	Date of analysis [dd-mm-yy]	Concentration		
			Nominal [mg/l]	Analysed [1] [mg/l]	Relative to nominal [%]
0 (fresh)	09-09-02	13-09-02 [2]	0	n.d.	n.a.
			10	9.58	96
		26-09-02 [2]	18	17.8	99
			32	31.4	98
			56	55.2	99
			100	101	101
24(old)	10-09-02	13-09-02 [2]	0	0.418	n.a.
			10	7.85	79
			18	13.9	77
			32	25.6	80
			56	48.5	87
72 (fresh)	12-09-02	13-09-02 [2]	0	0.150	n.a.
			10	9.83	98
			18	17.9	99
96 (fresh)	13-09-02	13-09-02	0	0.642	n.a.
			10	8.08	81
			18	16.2	90

[1] Mean of duplicate samples. The maximum deviation between the responses was calculated for each sample and was < 10%.

[2] Samples were frozen until analysis.

n.d. Not detected.

n.a. Not applicable.